



Reduction of foodborne micro-organisms on beef carcass tissue using acetic acid, sodium bicarbonate, and hydrogen peroxide spray washes

Kristen Y. Bell¹, Catherine N. Cutter² and Susan S. Sumner^{3*}

In an attempt to control beef carcass contamination, a search for effective carcass washing treatments has become a major focus in the area of microbiological meat safety. Spray-wash treatments utilizing 1.0% acetic acid, 3% hydrogen peroxide, 1% sodium bicarbonate, alone or in combination, were performed to evaluate their efficacy in reducing numbers of Escherichia coli, Listeria innocua and Salmonella wentworth. The fascia surface of lean and adipose tissue was inoculated with sterilized fecal slurry containing the designated bacteria to obtain $5 \log_{10}$ cfu cm^{-2} . A pilot scale model carcass washer was used to apply the spray treatment (80 psi, 15 s, 25°C). Control samples received no spray treatments. Following treatments, lean and adipose samples were immediately analyzed or held for 24 h at 5°C for analysis of the treatments, for residual bacterial populations, surface pH, color analysis, and residual hydrogen peroxide. The combination wash of acetic acid/3% hydrogen peroxide (AAHP) resulted in the greatest reductions of 3.97 and 3.69 \log_{10} cfu cm^{-2} for E. coli on lean or adipose tissue, respectively. Spray washes with AAHP reduced L. innocua by 3.05 \log_{10} cfu cm^{-2} on lean tissue and 3.52 \log_{10} cfu cm^{-2} on adipose tissue, while S. wentworth was reduced by 3.37 \log_{10} cfu cm^{-2} on lean and 3.69 \log_{10} cfu cm^{-2} on adipose tissue. A spray-wash treatment consisting of the right combination of safe and acceptable solutions may be effective for improving the microbial safety of beef.

© 1997 Academic Press Limited

Received: 10
November 1996

¹Department of Food Science and Technology, University of Nebraska—Lincoln, Lincoln, Nebraska 68583-0919,

²Roman L. Hruska U. S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, P. O. Box 166, Clay Center, Nebraska 68933, and

³Department of Food Science and Technology, Virginia Tech, Blacksburg, Virginia 24061-0418, USA

Introduction

The United States Department of Agriculture Food Safety and Inspection Service has proposed reforms for the meat and poultry industry in an attempt to reduce microbial contamination (Anonymous 1995). Requirements of the reform include the implementation of a Hazard Analysis Critical Control Point (HACCP) plan, microbial testing, and sanitation standard operating practices.

Microbial testing will focus on organisms such as *Salmonella* spp. and *Escherichia coli* (Anonymous 1995). In order to comply with the reforms and to provide microbiologically safe products to the consumer, methods for reducing the organisms listed above are being investigated by researchers and the meat industry.

Mechanical spray washing of carcasses was not actually considered by the meat industry until 1981 when Tarpoff and Swientek (1981) focused on the savings it could generate in labor and time. They calculated that the mechanical beef carcass washing

*Corresponding author.

system, which utilized water, reduced labor requirements and the time of washing from 105 s/half carcass (washing with hand held) to 15 s/half carcass (Tarpoff and Swientek 1981). Recently, the goal of carcass washing has been modified to incorporate a substance(s) in the spray solution that can be applied to the beef carcass surface to reduce microbial contamination.

Organic acids have been studied to determine their antimicrobial activity, and in 1982 acetic acid was approved as a sanitizer for beef carcasses (Federal Register 1982). Studies performed since that time have indicated that acetic acid is effective for reducing *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhimurium*, three organisms which are of major concern in the beef industry (Anderson et al. 1987, Dickson 1991, Dickson and Anderson 1991). Karapiran and Gonul (1992) investigated the effects of vinegar, sodium bicarbonate, acetic and citric acids on the growth of *Yersinia enterocolitica*. Hydrogen peroxide is an antimicrobial that can damage proteins, lipids, DNA, and cell membranes when it accumulates in a bacterial cell (Davis et al. 1990). Hydrogen peroxide has been used in the dairy industry to surface sterilize processing equipment and packaging materials (Davidson et al. 1983). It has also been considered in the poultry industry to decontaminate broiler carcasses (Lillard and Thomsen 1983, Mulder et al. 1987, Izat et al. 1989). Another decontamination method utilizing a baking soda solution (sodium bicarbonate) and hydrogen peroxide was patented (4 683 618) and claimed to remove bacteria and foreign matter from poultry carcasses (O'Brien 1987).

Currently, a limited number of studies incorporating combinations of organic acid spray washes have been published. In a study combining acetic, lactic, citric and ascorbic acids, researchers found the combination of 2.0% lactic acid, 1.0% acetic acid, 0.25% citric acid and 0.1% ascorbic acid produced reductions in bacterial populations (Dickson and Anderson 1992). Similarly, Garcia Zepeda et al. (1994) tested organic acid spray wash combinations of gluconic acid and lactic acid, and found the combinations to be more effective at reducing microbial popu-

lations than the use of these organic acids singularly. The objective of this project was to determine the efficacy of acetic acid, sodium bicarbonate and hydrogen peroxide treatments alone and in combination on the survival of micro-organisms inoculated onto lean and adipose beef tissue.

Materials and Methods

Escherichia coli ATCC 25922 obtained from American Type Culture Collection; *L. innocua* (R. L. Hruska U. S. Meat Animal Research Center, Clay Center, NE, USA RLHUSMARC); and *S. wentworth* (Steve Craven, USDA, ARS, Athens, GA, USA) were maintained in 75% glycerol at -20°C . They were cultured in tryptic soy broth (Troy-Biologicals, Troy, MI, USA) at 37°C for 24 h. Because of the risk of aerosol exposure during spray washing, these bacterial cultures were used as pathogen models.

Fecal material was collected from heifers/steers/cows/bulls located at the RLHUSMARC. Feces were stored at 0°C , thawed on the day of use, and a 1:1 fecal slurry was prepared with distilled water. This solution was sterilized for 20 min at 121°C and reconstituted up to original volume with sterile distilled water. The sterile fecal slurry was then inoculated with the appropriate organism to 10^7 cfu ml^{-1} .

Pre-rigor lean and adipose beef carcass tissue were obtained from a local packing plant, placed into plastic bags, stored in an insulated container, and transported back to the laboratory within 1 h post exsanguination. The lean tissue was removed from the cutaneous trunci and the adipose tissue was removed from the loin area. Tissues were trimmed to provide 7.5 cm \times 7.5 cm samples and surface sterilized with ultraviolet light (60 W germicidal bulbs, General Electric; 51 cm distance from tissue surface) for 20 min on each side (Cutter and Siragusa 1994). The fascia surface was brush inoculated with an inoculated fecal slurry containing either *E. coli* 25922, *L. innocua*, or *S. wentworth*, and incubated for 15 min at 25°C , (Dorsa et al. 1996) to obtain 5 log₁₀ cfu cm^{-2} .

Treatments and spray washing

Treatment categories were: untreated (U); sterile distilled water (W; pH 4.84); 1.0% acetic acid (AA; glacial, (v/v); Fisher, Pittsburgh, PA, USA; pH 2.92); 1.0% sodium bicarbonate (SB; (w/v); Sigma, St. Louis, MO, USA; pH 5.31); 3.0% hydrogen peroxide (HP; 3% (v/v); Baxter, St. Louis, MO, USA; pH 5.31); 1.0% acetic acid/3.0% hydrogen peroxide (AAHP); and 1.0% sodium bicarbonate/3.0% hydrogen peroxide (SBHP). All solutions were prepared in sterile distilled water the same day of use. A pilot scale model carcass washer located at the RLHUS-MARC was used to perform the spray washing (Cutter and Siragusa 1994). The parameters for wash were as follows: spray nozzle 25/1.0 (25° angle, 1 gallon min⁻¹ at 40 psi; Spraying Systems Co., Wheaton, IL, USA); oscillation speed 60 oscillations/min; line pressure 80 psi; flow rate 5.096 l min⁻¹. Distance from the nozzle to the tissue surface, 22 cm; and spray application=25°C. The samples treated with a single treatment were sprayed for 15 s, held for 90 s, and sprayed again with the same compound for 15 s. Samples treated with two compounds were subjected to the first compound for 15 s, held for 90 s, and sprayed with the second compound for 15 s.

Bacterial enumeration

Immediately after spray washing, all samples were aseptically trimmed to 5 cm×5 cm and placed in Stomacher bags (Tekmar, Cincinnati, OH, USA). The remaining trimmings from each sample were placed in Whirl-pak bags (Nasco, Fort Atkinson, WI, USA) and were used to determine surface pH values (flat electrode, Corning Instruments, Corning, NY, USA), Hunter color data (Minolta Chroma Meter 300 for Hunter Lab Color System, Ramsey, NJ, USA), and residual hydrogen peroxide (CHEMetrics, Calverton, VA, USA). Samples used for determining residual hydrogen peroxide values were diluted (1:100) in distilled water. This water solution was then sampled for colorimetric analysis using self-filling ampoules containing an acidic solution of ammonium thiocyanate. The manufacturer's testing

instructions were followed and samples were immediately analysed. Within the Stomacher bag, samples were pummeled (Stomacher 400, Tekmar, Cincinnati, OH, USA) for 2 min in 25 ml of 2.0% buffered peptone water (BPW) (Becton Dickinson, Cockeysville, MD, USA) with 0.1% Tween 20 (Fisher). Samples were serially diluted in 2% BPW, and dispensed on tryptic soy agar (Difco, Detroit, MI, USA) with a Model D Spiral Plater (Spiral Biosystems Instruments, Bethesda, MD, USA). Plates were incubated for 24 h at 37°C and enumerated using a Casba Image Analyzer (Spiral Biosystems Instruments).

Experimental design and statistical analyses

The experimental design was a seven treatments×3 bacterial strains×2 tissue types factorial design. Bacterial populations (cfu ml⁻¹) were obtained from three replications performed on separate days and converted to log₁₀ cfu cm⁻². Differences between log₁₀ cfu cm⁻² untreated beef carcass tissue and log₁₀ cfu cm⁻² treated beef carcass tissue were calculated as a log reduction. Log reductions of treatments were analysed by analysis of variance using the general linear models of SAS (SAS Institute, Cary, NC, USA). The probability level was *P*<0.05 unless otherwise noted.

Results

Log reductions

Analysis of variance (ANOVA) of log reductions associated with populations of *E. coli* indicated that treatment and tissue type were significant variables, but no two- or three-way interactions occurred. Of the individual treatments analysed, W, SB, HP, AA, SBHP, and AAHP affected overall reductions of 2.21, 2.29, 2.98, 3.04, 2.94, and 3.62 log₁₀ cfu cm⁻², respectively (Fig. 1). When tissue type was analysed by ANOVA, greater and statistically significant reductions were found on lean tissue (3.04 log₁₀ cfu cm⁻²) vs adipose tissue (2.65 log₁₀ cfu cm⁻²). Of the treatments investigated against *E. coli*, a

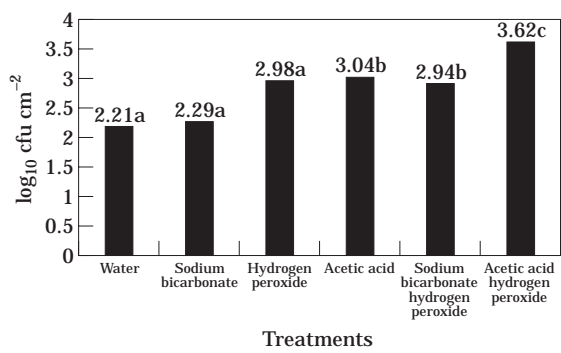


Figure 1. Mean log reductions (log cfu cm⁻²) of *Escherichia coli* ATCC 25922 on tissue after antimicrobial washes. ^{a-c}Denote statistical difference between treatments.

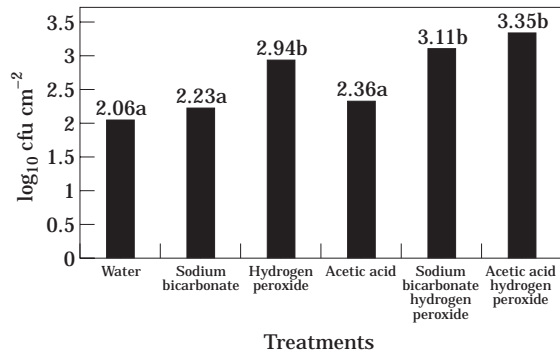


Figure 2. Mean log reductions (log cfu cm⁻²) of *L. innocua* on tissue after antimicrobial washes. ^{a-b}Denote statistical difference between treatments.

combination wash (AAHP) affected reductions of 2.92, 3.69 on adipose tissue at days 0 and 1 as well as reductions of 3.92 and 3.97 on lean tissue at days 0 and 1 (Table 1). ANOVA analyses of log reductions from populations of *L. innocua* demonstrated that only treatment was significant; no two- or three-way interactions occurred. Log

reductions of W, SB, HP, AA, SBHP, and AAHP were 2.06, 2.23, 2.94, 2.36, 3.11, and 3.35, respectively for the organism (Fig. 2). As indicated in Table 2, the combination washes (AAHP, SBHP) affected the greatest reductions of *L. innocua* on adipose tissue, while any treatment containing HP provided the greatest reductions on lean tissue.

Table 1. Mean log reductions (log cfu cm⁻²) of *Escherichia coli* ATCC 25922 on adipose and lean tissue after antimicrobial washes

Treatment	Adipose		Lean	
	Day 0 ^a	Day 1	Day 0	Day 1
Distilled water	1.96	2.33	2.33	2.22
1% Acetic acid	2.47	2.96	3.12	3.35
1% Sodium bicarbonate	1.96	2.03	2.55	2.61
3% Hydrogen peroxide	2.71	2.78	3.34	3.34
1% Acetic acid/3% hydrogen peroxide	2.92	3.69	3.92	3.97
1% Sodium bicarbonate/3% hydrogen peroxide	3.15	2.82	2.76	3.02

^aDay 0 depicts log reductions within 3 h after applying spray wash; day 1 depicts log reduction after 24 h storage at 5°C.

Table 2. Mean log reductions (log cfu cm⁻²) of *Listeria innocua* LA-1 on adipose and lean tissue after antimicrobial washes

Treatment	Adipose		Lean	
	Day 1	Day 0	Day 1	
Day 0 ^a				
Distilled water	2.07	1.86	2.45	1.87
1% Acetic acid	1.94	2.85	2.23	2.42
1% Sodium bicarbonate	1.58	2.46	2.43	2.46
3% Hydrogen peroxide	2.95	2.30	3.39	3.13
1% Acetic acid/3% hydrogen peroxide	3.05	3.52	3.79	3.05
1% Sodium bicarbonate/3% hydrogen peroxide	3.34	2.94	3.18	2.98

^aDay 0 depicts log reductions within 3 h after applying spray wash; day 1 depicts log reduction after 24 h storage at 5°C.

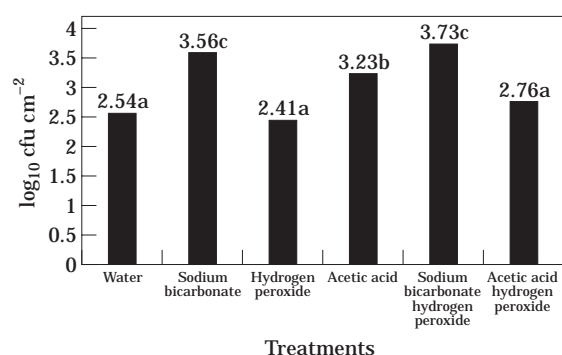


Figure 3. Mean log reductions (log cfu cm⁻²) of *Salmonella wentworth* on tissue after antimicrobial washes. ^{a-c}Denote statistical difference between treatments.

Treatment was the only significant variable when log reductions of *S. wentworth* were analysed. There were no two- or three-way interactions. Log reductions of W, SB, HP, AA, SBHP, and AAHP were 2.54, 3.56, 2.41, 3.23, 3.73, and 2.56, respectively (Fig. 3). As indicated in Table 3, the AA and AAHP washes affected the greatest log reductions of *S. wentworth* on adipose tissue, while the AA, HP, and AAHP wash treatments provided the greatest log reductions on lean tissue.

pH effect

When compared with data from day 0, the surface pH values of the lean tissue at day 1 returned to a pH range of 5.91–6.43 (Table 4) after spray washing with AA, HP, and AAHP. The pH range of adipose tissue at day 1 was 5.24–7.81 and was dependent upon treatment. Of the treatments tested, AAHP reduced the surface pH the greatest on both

tissues at day 0 and day 1. As indicated by statistical analyses of pH data for tissues inoculated with *E. coli*, treatment, tissue, and day were significant variables; treatment×tissue and treatment×day were significant two-way interactions. Surface pH values from tissue inoculated with *L. innocua* yielded significant variables of treatment and tissue; and one two-way interaction: treatment×tissue. As demonstrated with *E. coli*, the AAHP treatment reduced surface pH values the greatest on lean and adipose tissues at days 0 and 1 (Table 5). Unlike pH data of *E. coli* and *L. innocua*, data analyses of surface pH data of tissues inoculated with *S. wentworth* demonstrated that treatment, tissue, and day were significant variables; treatment×tissue; treatment×day; and tissue×day were significant two-way interactions; and treatment×tissue×day was a significant three-way interaction (Table 6). Of the treatments, AA and AAHP reduced surface pH values on lean and adipose tissues on day 0 and 24 h of refrigerated storage. Generally, beef surfaces treated with acetic acid (AA) alone or in combination with hydrogen peroxide (AAHP), affected the greatest drop in pH values at day 0, regardless of organism tested. After 24 h, pH values of surfaces treated with AA alone or AAHP increased slightly, but not to the values demonstrated by U, or tissue treated with W, HP, SB or SBHP.

Color effects and residual hydrogen peroxide

The Hunter L, a, and b values obtained from all the tissues in these experiments are pre-

Table 3. Mean log reductions (log cfu cm⁻²) of *Salmonella wentworth* adipose and lean tissue after antimicrobial washes

Treatment	Adipose		Lean	
	Day 0 ^a	Day 1	Day 0	Day 1
Distilled water	2.48	2.27	2.77	2.64
1% Acetic acid	3.51	3.70	3.47	3.66
1% Sodium bicarbonate	2.30	2.79	2.24	2.33
3% Hydrogen peroxide	2.66	3.39	3.51	3.35
1% Acetic acid/3% hydrogen peroxide	3.83	3.69	3.65	3.73
1% Sodium bicarbonate/3% hydrogen peroxide	2.57	2.76	2.60	2.31

^aDay 0 depicts log reductions within 3 h after applying spray wash; day 1 depicts log reduction after 24 h storage at 5°C.

sented in Table 7. When compared with the untreated sample, both the treated lean and adipose tissues had a minimal increase in Hunter L values which indicates an increase in the lightness of the sample. In general, the Hunter a values of the lean and adipose tissues decreased for each of the treatments which corresponds to a decrease in the red color of the two tissue types. The yellow pigments or Hunter b values also decreased for the treated lean and adipose tissue compared with the untreated samples.

The average residual hydrogen peroxide level for all tissues treated with AAHP was

1.4 ppm and the average residual hydrogen peroxide level for water treated carcasses was 0.5 ppm (data not shown).

Discussion

The main goal of this project was to find a spray wash treatment that decreased microbial loads of bacteria on beef tissue. To date, organic acids have been found to somewhat satisfy these standards (Hardin et al. 1995, Cutter and Siragusa 1994, Anderson et al. 1987); however, greater microbial

Table 4. Average surface pH values after spray wash treatments for beef carcass tissue, lean and adipose, inoculated with *Escherichia coli* ATCC 25922

Treatment	Tissue type	pH (Day 0)	pH (Day 1)
Untreated	L	6.53	6.10
Distilled water	L	6.49	6.12
1.0% Acetic acid	L	5.02	5.67
3.0% Hydrogen peroxide	L	6.20	5.91
1.0% Sodium bicarbonate	L	7.23	6.66
1.0% Acetic acid/3.0% hydrogen peroxide	L	5.52	5.69
1.0% Sodium bicarbonate/3.0% hydrogen peroxide	L	6.98	6.43
Untreated	A	7.17	6.89
Distilled water	A	7.02	6.90
1.0% Acetic acid	A	4.49	4.61
3.0% Hydrogen peroxide	A	6.98	6.84
1.0% Sodium bicarbonate	A	8.22	7.87
1.0% Acetic acid/3.0% hydrogen peroxide	A	5.20	5.24
1.0% Sodium bicarbonate/3.0% hydrogen peroxide	A	7.71	7.81

Table 5. Average surface pH values after spray wash treatments for beef carcass tissue, lean and adipose, inoculated with *Listeria innocua* LA-1

Treatment	Tissue Type	pH (Day 0)	pH (Day 1)
Untreated	L	6.34	6.08
Distilled water	L	6.32	6.08
1.0% Acetic acid	L	5.10	5.56
3.0% Hydrogen peroxide	L	6.29	6.08
1.0% Sodium bicarbonate	L	7.25	6.32
1.0% Acetic acid/3.0% hydrogen peroxide	L	5.42	5.69
1.0% Sodium bicarbonate/3.0% hydrogen peroxide	L	7.19	6.40
Untreated	A	6.97	7.18
Distilled water	A	6.64	6.79
1.0% Acetic acid	A	4.61	4.56
3.0% Hydrogen peroxide	A	7.39	7.05
1.0% Sodium bicarbonate	A	8.05	7.85
1.0% Acetic acid/3.0% hydrogen peroxide	A	5.30	5.26
1.0% Sodium bicarbonate/3.0% hydrogen peroxide	A	8.00	8.07

reductions are still desired. While *E. coli* and *L. innocua* were chosen as pathogen models in this study, no attempt was made to determine the impact that strain difference would have on removal of undesirable bacteria from beef. Pre-rigor tissue used 60–90 min post exsanguination, which was used in this study, may not represent normal processing, however, this tissue type may be the only tissue that scientists have to access in laboratory settings or pilot plant conditions.

In the present study, AAHP spray combination consistently produced the greatest log reductions, regardless of the organism or the tissue type (an average reduction of 3.57 logs). Antimicrobial washes consisting of AAHP can provide better reductions of undesirable bacteria on beef surfaces, as compared with water washing alone. This observation may be attributable to the synergistic effect of organic acids in combination with hydrogen peroxide. The AAHP combination is analogous to a peroxyacetic acid sanitizer. It is possible that the oxidizing effect occurring on the tissue samples is also enhanced when the surface pH is decreased following the application of acetic acid. The spray washes with a more neutral pH, including HP and SBHP, were generally less effective than AAHP at reducing microbial loads on the beef tissue.

Unlike previous studies (Dickson 1991, Cutter and Siragusa 1994), in which the adipose tissue was treated with acetic acid and

resulted in the largest reductions, the lean tissue had the greater average log reductions in this study. Statistical analysis indicated that tissue type did affect log reductions of *E. coli* but not for *L. innocua* and *S. wentworth*. Another reason for differences between the studies may be the acetic acid used. Other studies indicated that the greater the acid concentration, the greater the difference in log reductions between tissue types (Dickson 1991, Cutter and Siragusa 1994). The present study employed 1.0% acetic acid, which may not be concentrated enough to consistently produce significant differences between tissue types. Previous research has reported that the pH decrease on adipose tissue following an acidic spray was greater than that on lean tissue (Dickson 1992). The present study supports this observation. The factor(s) responsible for this pH phenomenon has not been specifically targeted or explained. One possibility is that the lean tissue continues to produce lactic acid following slaughter which causes its surface pH value to equilibrate at a higher common value (Pearson 1987). Adipose tissue does not contain the same level of glycogen as lean tissue so it is not able to produce lactic acid via glycolysis (Pearson 1987).

The antimicrobial activity of hydrogen peroxide is not disputed. It has been used for sterilizing equipment and packaging in the food industry, especially in the dairy sector for many years (Davidson et al. 1983). Its

Table 6. Average surface pH values after spray wash treatments for beef carcass tissue, lean and adipose, inoculated with *Salmonella wentworth*

Treatment	Tissue Type	pH (Day 0)	pH (Day 1)
Untreated	L	6.37 ^b	5.89 ^b
Distilled water	L	6.44 ^b	5.97 ^b
1.0% Acetic acid	L	4.94 ^a	5.23 ^a
3.0% Hydrogen peroxide	L	6.12 ^b	5.95 ^b
1.0% Sodium bicarbonate	L	7.44 ^c	6.13 ^c
1.0% Acetic acid/3.0% hydrogen peroxide	L	5.23 ^a	5.60 ^a
1.0% Sodium bicarbonate/3.0% hydrogen peroxide	L	7.21 ^c	6.23 ^b
Untreated	A	6.63 ^c	6.55 ^c
Distilled water	A	7.27 ^d	6.92 ^c
1.0% Acetic acid	A	4.25 ^a	4.60 ^a
3.0% Hydrogen peroxide	A	7.29 ^d	6.78 ^c
1.0% Sodium bicarbonate	A	8.17 ^e	8.44 ^e
1.0% Acetic acid/3.0% hydrogen peroxide	A	4.91 ^b	5.08 ^b
1.0% Sodium bicarbonate/3.0% hydrogen peroxide	A	7.57 ^d	7.59 ^d

^{a-e}Denote statistical difference between treatments with day and tissue type.

Table 7. Hunter L, a, and b values at days 0 and 1 for lean and adipose beef tissues inoculated with bacteria and subjected to spray treatments

Treatment	Day	Escherichia coli						Listeria innocua						Salmonella wentworth					
		Lean			Adipose			Lean			Adipose			Lean			Adipose		
		L	a	b	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Untreated	0	39.14	8.49	9.88	53.88	6.96	27.76	37.70	4.77	8.17	53.67	3.64	20.73	44.57	9.51	15.68	57.35	2.69	28.85
Distilled Water	0	45.46	7.66	6.15	61.77	5.81	22.18	52.17	4.66	7.21	64.18	2.24	23.25	44.48	10.12	6.22	59.76	7.61	27.77
1% Sodium bicarbonate	0	49.86	5.49	4.00	62.44	3.17	26.49	49.97	6.31	4.60	56.45	6.31	30.66	44.75	8.33	4.20	58.55	4.31	28.10
3% Hydrogen peroxide	0	51.22	3.95	7.37	57.57	3.14	25.66	52.53	6.85	4.43	64.78	6.85	29.17	46.24	3.85	8.19	64.02	6.01	24.44
1% Acetic acid	0	47.50	5.62	5.71	60.85	1.19	20.87	50.58	5.79	12.05	57.24	5.79	20.99	47.13	5.52	15.31	63.26	1.58	25.79
1% Sodium bicarbonate, 3% hydrogen peroxide	0	56.52	3.03	12.31	71.17	2.88	17.24	56.82	5.79	3.69	56.45	5.27	27.21	49.41	6.04	5.50	64.32	4.02	25.08
1% Acetic acid, 3% hydrogen peroxide	0	54.67	5.73	13.12	61.8	0.39	26.68	51.03	7.34	7.80	64.28	3.42	19.02	55.27	5.46	9.50	59.47	4.49	25.83
Untreated	1	38.48	9.25	9.44	54.70	4.06	28.69	38.75	7.28	10.80	54.29	5.00	24.08	38.80	6.74	12.75	55.59	4.53	28.68
Distilled water	1	53.61	6.20	12.13	62.44	4.56	23.22	50.28	8.53	10.97	62.90	6.19	22.23	45.35	8.16	6.63	64.16	4.89	27.43
1% Sodium bicarbonate	1	47.89	5.76	3.00	61.64	3.56	21.80	51.77	4.61	4.83	61.59	4.62	29.54	46.65	7.83	4.41	60.06	4.58	26.32
3% Hydrogen peroxide	1	48.13	5.19	6.56	63.88	3.50	25.76	45.03	8.14	3.85	67.24	8.14	23.82	53.11	4.70	10.98	64.46	3.40	22.81
1% Acetic acid	1	54.87	3.85	11.30	62.68	0.12	25.16	54.97	4.90	14.94	62.99	4.10	21.66	55.43	4.45	14.28	64.22	1.36	24.33
1% Sodium bicarbonate, 3% hydrogen peroxide	1	48.99	5.71	3.75	68.31	2.07	15.92	59.06	1.18	5.21	61.59	3.91	16.98	54.02	6.08	10.32	63.22	3.73	28.23
1% Acetic acid, 3% hydrogen peroxide	1	56.69	4.26	10.19	61.56	1.93	22.72	54.78	3.44	10.55	66.93	0.46	19.85	54.32	6.16	11.77	66.13	1.00	29.92

direct applications to poultry raised questions concerning bleaching of the carcass (Lillard and Thomsen 1983, Mulder et al. 1987, Izat et al. 1989). The data compiled in this study indicates that the most effective spray wash, AAHP, did not produce significant changes in the Hunter L or Hunter b values of either tissue type. The red pigments, Hunter a, in both tissue types showed the largest decrease.

A second aspect of concern regarding the use of hydrogen peroxide on beef carcass tissue involves the amount of residual hydrogen peroxide remaining on the carcass surface. The tests for residual hydrogen peroxide on the beef carcass tissue indicated that at or near neutral pH, the catalase present in the tissue can sufficiently convert the hydrogen peroxide to water and oxygen leaving no residual hydrogen peroxide on the surface. Under acidic conditions (AAHP), it appears the catalase is possibly inhibited, and therefore, less efficient in breaking down the hydrogen peroxide. Because this spray-wash treatment was the most effective at reducing the microbial load, further research regarding the residual activity of hydrogen peroxide on the tissue is justified.

The findings of this study suggest that the combination of acetic acid with hydrogen peroxide administered through spray washing may provide a powerful tool for reducing microbial contamination on beef carcasses. Incorporation of such a combination of treatments may satisfy the requirements of the meat industry and the government to reduce the number of bacteria on meat carcasses. Additional investigation into the effectiveness of combination washes may result in more microbial contamination control options for meat processors.

Acknowledgements

The authors wish to thank Laura Albee and Dana Schnoor for their technical assistance, and Jim Wray for statistical analyses support. We would also like to thank Lovett and Sons Packing Company of Hastings, NE for their co-operation. Funding was made possible by the University of Nebraska Institute

of Agriculture and Natural Resources project #NEB-16-058.

References

- Anderson, M. E., Huff, H. E., Naumann, H. D., Damare, J. M., Pratt, M., Johnson, R. and Marshall, R. T. (1987) Evaluation of an automated beef carcass washing and sanitizing system under production conditions. *J. Food Protect.* **50**, 562–566.
- Anonymous, (1995) USDA releases proposal to reform meat and poultry inspection rules. *World Food Reg. Rev.* **4**, 20–21.
- Cutter, C. N. and Siragusa, G. R. (1994) Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using pilot scale model carcass washer. *J. Food Protect.* **57**, 97–103.
- Davidson, P. M., Post, L. S., Branen, A. L. and McGurdy, A. R. (1983) Naturally occurring and miscellaneous food antimicrobials. In *Antimicrobials in Foods*. (Eds Branen, A. L. and Davidson, P. M.) pp. 371–419. New York, Marcel Dekker, Inc.
- Davis, B. D., Dulbero, R., Eisen, H. N. and Jinsberg, H. S. (1990) Cell mediated immunity. In *Microbiology*. pp. 431–452. Philadelphia, J. B. Lippincott Co.
- Dickson, J. S. (1991) Attachment of *Salmonella typhimurium* and *Listeria monocytogenes* to beef tissue: effects of inoculum level, growth temperature and bacterial culture age. *Food Microbiol.* **8**, 143–151.
- Dickson, J. S. (1992) Acetic acid action on beef tissue surfaces contaminated with *Salmonella typhimurium*. *J. Food Sci.* **57**, 297–301.
- Dickson, J. S. and Anderson, M. E. (1991) Control of *Salmonella* on beef tissue surfaces in a model system by pre- and post-evisceration washing and sanitizing, with and without spray chilling. *J. Food Protect.* **54**, 514–518.
- Dickson, J. S. and Anderson, M. E. (1992) Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. *J. Food Protect.* **55**, 133–140.
- Dorsa, W. J., Cutter, C. N., Siragusa, G. R. and Koohmarie, M. (1996) Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. *J. Food Protect.* **59**, 127–135.
- Federal Register*, Vol. 47, No. 123, Friday, June 25, 1982. Rules and Regulations, Part 184.
- Garcia Zepeda, C. M., Kastner, C. L., Willard, B. L., Phebus, R. K., Schwenke, J. R., Fijal, B. and Prasai, R. K. (1994) Gluconic acid as a fresh beef decontaminant. *J. Food Protect.* **57**, 956–962.
- Hardin, M. D., Acuff, G. R., Lucia, L. M., Oman, J. S. and Savell, J. W. (1995) Comparison of

- methods for decontamination from beef carcass surfaces. *J. Food Protect.* **58**, 368–374.
- Izat, A. L., Colberg, M., Adams, M. H., Reiber, M. A. and Waldroup, P. W. (1989) Production and processing studies to reduce the incidence of *Salmonellae* on commercial broilers. *J. Food Protect.* **52**, 670–673.
- Karapinar, M. and Gonul, S. A. (1992) Effects of sodium bicarbonate, vinegar, acetic and citric acids on growth and survival of *Yersinia enterocolitica*. *Int. J. Food Microbiol.* **16**, 343–347.
- Lillard, H. S. and Thomsen, J. E. (1983) Efficacy of hydrogen peroxide as a bactericide in poultry chiller water. *J. Food Sci.* **48**, 125–126.
- Mulder, R. W. A. W., van der Hulst, M. C. and Bolder, N. M. (1987) Research note: *Salmonella* decontamination of broiler carcasses with lactic acid, L. cysteine, and hydrogen peroxide. *Poultry Sci.* **66**, 1555–1557.
- O'Brien, G. T. (1987) Reduction of bacteria count on poultry being processed into food at a poultry processing plant. U. S. Patent No. 4 683 618 August 4, 1987.
- Pearson, A. M. (1987) Muscle function and post-mortem changes. In *The Science of Meat and Meat Products*. (Eds Price, J. F. and Schweigert, B. S.) pp. 155–192. Westport, CT, Food and Nutrition Press, Inc.
- Price, J. F. and Schweigert, B. S. (1987) *The Science of Meat and Meat Products*. 3rd edn Westport, CT, Food and Nutrition Press.
- Pruitt, K. M. and Reitter, B. (1985) Biochemistry of peroxidase system: antimicrobial effects. In *The Lactoperoxidase System*. (Eds Pruitt, K. M. and Tenovuo, J. O.) pp. 143–178. New York, Marcel Dekker.
- Tarpoft, J. and Swientek, R. J. (1981) Beef carcass washing system cuts cleaning time 85%. *J. Food Protect.* **42**, –82.